GROWTH IN WEIGHT AND OF SOME TISSUES IN THE BULLFROG: FITTING NONLINEAR MODELS DURING THE FATTENING PHASE

Crescimento em peso e de alguns tecidos de rã-touro: através de modelos não lineares durante a fase de engorda

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ABSTRACT

Knowledge of the growth of animals is important so that zootechnical activity can be more accurate and sustainable. The objective of this study was to describe the live weight, development of liver tissue and fat body, leg growth, and cumulative food intake of bullfrogs during the fattening phase using nonlinear models. A total of 2,375 bullfrog froglets with an initial weight of 7.03 ± 0.16 g were housed in five fattening pens (12 m^2). Ten samplings were performed at intervals of 14 days to obtain the variables studied. These data were used to estimate the parameters of Gompertz and logistic models as a function of time. The estimated values of weight (W_m) and food intake (FI_m) at maturity and time when the growth rate is maximum (t*) were closer to expected values when the logistic model was used. The W_m values for live weight and liver, adipose and leg weights and the FI_m value for food intake were 343.7, 15.7, 19.6, 96.03 and 369.3 g, respectively, with *t** at 109, 98, 105, 109 and 107 days. Therefore, the logistic model was the best model to estimate the growth and food intake of bullfrogs during the fattening phase.

Index terms: Frog farming, growth curves, growth rate, logistic model.

RESUMO

O conhecimento do crescimento dos animais é importante para a atividade zootécnica ser mais precisa e sustentável. O objetivo do estudo foi descrever o crescimento em peso vivo, dos tecidos hepático e adiposo, da coxa e o consumo alimentar acumulado da rã-touro na engorda, através de ajuste de modelos não lineares. Foram utilizados 2.375 imagos de rã-touro com peso inicial de 7,03±0,16g, os quais foram alojados em cinco baias de engorda com 12 m^2. Foram realizadas 10 amostragens, a cada 14 dias, para obtenção das variáveis estudadas. Os dados amostrados foram utilizados para estimar os parâmetros dos modelos de Gompertz e Logístico em função do tempo. Os valores estimados de peso (Pm) e consumo à maturidade (Cm) e tempo em que a taxa de crescimento é máxima (t*) dos parâmetros avaliados foram mais próximos do esperado no modelo Logístico. Os valores de Pm para peso vivo, tecido hepático e adiposo, coxas e Cm para consumo alimentar foram 343,7 g; 15,7g; 19.6g; 96,03 e 369,3g, respectivamente, com t* aos 109, 98, 105, 109 e 107 dias, respectivamente. Portanto, o modelo Logístico foi a melhor ferramenta para estimar o crescimento e o consumo alimentar da rã-touro na fase de engorda.

Termos para indexação: Ranicultura, curvas de crescimento, taxa de crescimento, logístico.

INTRODUCTION

Frog farming is an aquaculture activity of little socioeconomic impact in Brazil; as a consequence, investment in technology and in the development of supply industries is low. However, frog farming has an enormous potential considering the increase in the consumption of white and healthy meat by the population and also as an alternative source of protein (Mello et al. 2006).

Mathematical modeling is used in livestock production to assist technicians and researchers with the elaboration of animal breeding and nutrition programs in order to make zootechnical activities more accurate, profitable and sustainable. The most commonly used nonlinear mathematical equations to describe the weight gain of production animals are the Gompertz, logistic, Von Bertalanffy, Brody and Richards models. These equations are adopted because they contain three or more parameters that can be interpreted in biological terms (Santos et al. 2007).

Several studies have used nonlinear curves to describe the growth of different animal species such as cattle (Silva et al. 2011), sheep (Silva et al. 2012), buffaloes (Araújo et al. 2012), birds (Sakomura et al. 2011), fish (Dumas et al. 2010; Gomiero et al. 2009), and frogs in the terrestrial phase (Rodrigues et al. 2007) and aquatic phase of life (Mansano et al. 2012). Gompertz and logistic equations have been chosen as models to describe the

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growth of bullfrogs in the terrestrial phase (Rodrigues et al. 2007). However, the animals of that study were reared in the laboratory.

In addition to permitting the determination of body growth as a function of time, growth curves can be constructed to describe the growth of organs of the body (Marcato et al. 2010) and of commercial cuts (Marcato et al. 2009). In bullfrogs, important tissues include the liver because of its importance for good function of animal metabolism (Seixas Filho et al. 2009), and the fat body because of its ability to store fat during periods of low temperatures and reproduction (Pereira et al. 2011). The legs of the bullfrog, the edible part of the animal, consist of muscles (Fragoso et al. 2013) and bones (Bercu et al. 2012). The international trade of frog legs has an estimated value of 40 million dollars per year (Turnipseed et al. 2012). The world's largest consumers of frog legs are France and the United States (Neveu, 2009).

Zootechnical parameters such as food intake are important tools to evaluate livestock production. For pigs, daily digestible energy intake is related to body weight and can be estimated using empirical growth models (Hua et al. 2010). In fish, the accurate prediction of dietary intake is difficult due to biological and environmental factors (e.g., water and ambient temperature) and only few attempts have been made to use prediction models for the estimation of food intake in these animals (Hua et al. 2010). There are also no models to predict food intake in frogs and the same explanations as those reported for fish apply.

Little is known about the growth of bullfrogs and further studies providing additional data are needed. Therefore, the objective of the present study was to describe the live weight, development of liver tissue and fat body, leg growth, and cumulative food intake of bullfrogs during the fattening phase using nonlinear models.

MATERIAL AND METHODS

Local

The study was conducted at the Aquaculture Center of the Paulista State University (Universidade Estadual Paulista – UNESP), Sector of Raniculture, Jaboticabal, São Paulo, Brazil.

Animals, experimental conditions and sampling

A total of 2,415 bullfrog froglets (*Lithobates catesbeianus*) with a mean live weight (± standard error) of 7.03 ± 0.16 g were used. Forty animals were employed in the first sampling and the remaining 2,375 animals were housed in five fattening pens (12 m^2) (Lima, 1997) containing shelters, a central water trough, and vibrating feeders in a linear arrangement. Continuous water flow was provided from an artesian well.

The frogs received daily commercial extruded diet containing 39.65% crude protein, 4,366.3 kcal/kg crude energy, 4.89% crude fiber, and 10.28% mineral matter. Leftovers in the feeders were removed, dried in an oven at 55°C for 24 hours, and weighed for the calculation of food intake of the animals. The size of the pelleted ration ranged from 2 to 4 mm during the first 45 days and from 6 to 8 mm thereafter. The small water troughs of the pens were emptied and cleaned daily and the water was changed. Dead animals were removed.

The water and ambient temperature was measured with a minimum/maximum thermometer placed 30 cm from the floor, with the sensor being attached to the apparatus inside the water.

Ten samplings were performed at intervals of 14 days. Forty frogs were selected in the first sampling, 40 animals/pen in the second and third sampling, and 20 animals/pen in the fourth to tenth sampling. The frogs selected were stunned on ice and weighed on a digital scale to the nearest 0.01 g. Next, the spine was sectioned and the celomatic cavity was opened for the removal and weighing of liver and adipose tissue. The skin was also removed and the legs were cut and weighed.

The procedures adopted were approved by the Ethics Committee on the Use of Animals of the School of Agricultural and Veterinary Sciences, UNESP (Protocol No. 024999/10) and were conducted in accordance with the ethical guidelines of the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal - COBEA).

Statistical analyses

A completely randomized design consisting of five experimental units (12-m^2 pens) was used. The repetitions were the means of the five pens obtained in the 10 samplings. Mean live weight of the animal (g), liver weight (g), fat body weight (g) and leg weight (g) were used to describe the growth of bullfrogs, expressed as weight (g) as a function of age (days), using nonlinear Gompertz (Winsor, 1932) and logistic (Reed; Pearl, 1927) growth functions.

The following models were adopted to describe the growth curves:

Gompertz: $Wt = Wme^{-e^{-b(t-t^*)}}$ and

Logistic: $Wt = Wm / (1+e^{-b(t-t^*)})$,

where Wt = weight (g) at time t, estimated as a function of Wm; Wm = weight (g) at maturity; b = growth rate at maturity (g/day); t^* = time (days) when the growth rate is maximum; t = time (days). The estimated equation was then used to calculate the growth rates (g/day) as a function of time (t) by derivation of the Gompertz and logistic equations: $dWt'/dt = Wmbe^{-b(t-t^*)-e^{-b(t-t^*)}}$ and $dWt'/dt = b(Wt^2/Wm)e^{-b(t-t^*)}$, respectively.

The following equations were used to describe the cumulative food intake curve:

Gompertz: $FIt = FIme^{-e^{-b(t-t^*)}}$ and

logistic: $FIt = FIm/(1+e^{-b(t-t^*)})$,

where FIt = food intake (g) of the animal at time t, expressed as a function of FIm; FIm = cumulative food intake (g) at maturity; b = intake rate (g/day); t^* = time (days) when the intake rate is maximum; t = time (days). The parameters of the estimated equations were used to calculate daily food intake (g/day) as a function of time (t) by derivation of the equations.

The NLIN procedure of the Statistical Analysis System (SAS, 2008) software was used for parameter estimation. The parameter estimates were obtained by a modified iterative Gauss-Newton method developed by Hartley (1961) for nonlinear models.

The following criteria were used for selection of the most adequate model: residual mean square (RMS); Akaike information criterion (AIC) (Akaike, 1974), and mean absolute deviation of residuals (MAD) (Sarmento et al., 2006). The lower the value of MAD, the better the fit of the model. Linear regression was performed between observed values and values predicted with the best model (selected according to the criteria described above) for live weight and food intake using the PROC REG procedure of the SAS software (2008).

RESULTS AND DISCUSSION

The average maximum and minimum ambient temperatures recorded inside the facility were 33.35°C ± 3.20 and 21.26°C ± 1.69, respectively, with a mean difference (± standard deviation) of 12.09°C ± 3.46 between these temperatures. The difference between the maximum and minimum water temperature of the troughs in the pens was 3.01°C ± 1.80, with a maximum temperature of 30.95°C ± 0.62 and a minimum temperature of 27.94 °C ± 1.88.

At the end of the experimental period (126 days), the frogs had a mean live weight of 214.56 g; the animals had thus reached the cut-off weight of 200 g established for slaughter. In frog farming, the time of rearing the froglet until it reaches slaughter weight can vary from 77 (Borges et al. 2012) to 166 days (Teodoro et al. 2005). The main factor influencing the growth of frogs is the ambient temperature which exerts a direct effect on the metabolism of the animal; as observed for all anuran amphibians, the body temperature of bullfrogs depends on the temperature of their environment (Petersen; Gleeson, 2011).

Only the maximum and minimum ambient and water temperatures were recorded, a routine method in frog experiments. However, future studies should evaluate the duration of temperatures in an attempt to quantify the extent of the effect of high and low temperatures. In addition, the behavior and locomotion of frogs inside the pens at times of thermal discomfort should be monitored over a period of 24 hours. For example, in the afternoon when the temperatures were high, the frogs were found inside the water trough where temperatures were milder. There are other factors that can also influence the growth of frogs, such as initial animal weight (Álvarez; Real, 2006) and whether the animals are adapted to ration (Real et al., 2005).

With respect to the variables studied (live weight, liver weight, fat body weight, leg weight, and cumulative food intake), lower RMS (10.1960, 0.1501, 0.1002, 1.0227 and 18.9616, respectively), MAD (0.0294, 0.0462, 0.0689, 0.0076 and 0.3748), and AIC values (3.2022, 5.0002, 1.6200, 2.0978 and 1.3402) were obtained with the logistic model, indicating that this model better fits the data and should therefore be adopted (Table 1).

Although the differences in MAD values between the models were minimal (Table 1), this method can be used to select the model that presents the lowest value to fit the mean growth curve (Sarmento et al., 2006).

It should be noted that the results of studies designed to develop equations to describe the growth of amphibians depend on the species studied and the conditions tested (HOTA, 1994). The logistic model is indicated to describe the growth of animals over short periods of time (days and months) and in environments where some factors such as nutrition are controlled (Gamito, 1998).

The parameters of the logistic model (Wm and t*) estimated for the variables studied were close to the expected values, with Wm values of 343.7, 15.8, 19.7 and 96.9 g for live weight, liver weight, fat body weight and leg weight, respectively, with t^* (time when the growth rate is maximum) at 109, 98, 105 and 109 days, respectively (Table 1).

The values of Wm and t^* estimated with the Gompertz model for live weight (1,051.5 g and 177 days, respectively) and leg weight (300.7 g and 176.7 days) were high considering the period studied (Table 1). Bullfrog specimens reach this value along their life at more than 2 years of age. However, the value estimated for *Wm* with the logistic model (343.7 g) was adequate for the fattening period of froglets until they reach slaughter weight, with the frogs presenting a mean weight of 214.56 g at 126 days.

The values of *Wm* and *t** estimated with the Gompertz model for liver weight (38.13 g and 140 days) and fat body weight (54.04 g and 149 days) were also high and showed the same trend and interpretation as the results obtained for live weight and leg weight.

The *FIm* value estimated with the logistic model (369.3 g) was lower than that obtained with the Gompertz model (1,059.6 g). The period during which maximum food intake occurred (t*) was 107 days for the logistic model. This value is within the 126 days of bullfrog fattening and is thus closer to reality. Parameter *t** estimated with the Gompertz model was 156 days (Table 1).

Using the logistic model, *t** estimated for food intake (107 days) was similar to *t** estimated for live weight (109 days). This finding is important since it permits the use of food intake as a parameter to estimate the point of maximum growth of bullfrogs.

The values of the rate at maturity (b) estimated with the logistic model were 0.0313 g/day for live weight, 0.0373 g/day for liver weight, 0.0324 g/day for leg weight, and 0.0335 g/day for cumulative food intake. The highest values was observed for fat body weight (0.0430 g/day). This finding might be related to the ability of the animal to accumulate fat reserves during periods of low temperatures and reproduction (Pereira et al. 2011).

The value of *b* estimated for live weight with the logistic model (0.0313 g/day) was higher than the 0.0215 g/day reported by Rodrigues et al. (2007) for bullfrogs fed diets containing 42 to 52% crude protein, and lower than the 0.112 g/day estimated with a logistic model to describe the weight gain of bullfrog tadpoles (Mansano et al., 2012), confirming the rapid growth of tadpoles over a short period of time compared to frogs in the fattening stage.

Division of the food intake rate at maturity (b) by the live weight rate at maturity yields a value of 1.07, which corresponds to the estimated feed conversion at maturity.

The logistic model tended to estimate lower initial values than the Gompertz model, underestimating initial live weight on average by 4.12 g (Figure 1a). This trend has also been observed for bullfrogs reared in mini-paddock pens for 294 days beyond slaughter weight, in which the logistic model underestimated initial weight by 21.8 g (Rodrigues et al. 2007).

Table 1 – Parameter estimates and standard error, residual mean square (RMS), mean absolute deviation (MAD), and Akaike information criterion (AIC) obtained with the two models studied for live weight, tissue weights, leg weight and food intake of bullfrogs during the fattening phase.

Model	Wm (g)	b (g/day)	t* (days)	RMS	MAD	AIC
			Live weight			
Gompertz	1051.5 ± 82.1	0.0088 ± 0.002	177.6 ± 35.7	15.013	0.517	3.210
Logistic	343.7 ± 34.9	0.0313 ± 0.002	109.5 ± 6.8	10.196	0.029	3.202
			Liver weight			
Gompertz	38.2 ± 17.1	0.0113 ± 0.003	140.7 ± 36.1	0.249	0.049	5.395
Logistic	15.8 ± 1.8	0.0373 ± 0.004	98.7 ± 7.1	0.150	0.046	5.002
			Fat body weight			
Gompertz	54.1 ± 24.8	0.0123 ± 0.003	149.7 ± 32.6	0.217	0.071	1.663
Logistic	19.7 ± 2.1	0.0430 ± 0.004	105.2 ± 5.7	0.100	0.069	1.620
			Leg weight			
Gompertz	300.7 ± 119.5	0.0091 ± 0.002	176.7 ± 36.1	1.506	0.185	2.132
Logistic	96.9 ± 10.0	0.0324 ± 0.002	109.4 ± 6.7	1.022	0.008	2.098
	FIm (g)			Food intake		
Gompertz	1.059.6 ± 259.1	0.0102 ± 0.001	156.7 ± 23.2	19.167	2.195	1.745
Logistic	369.3 ± 35.4	0.0335 ± 0.002	107.5 ± 6.2	18.961	0.375	1.340

Wm = weight (g) at maturity; FIm = cumulative food intake (g) at maturity; b = growth rate or rate of food intake (g/day) at maturity; t* = time (days) when the growth rate or rate of food intake is maximum.

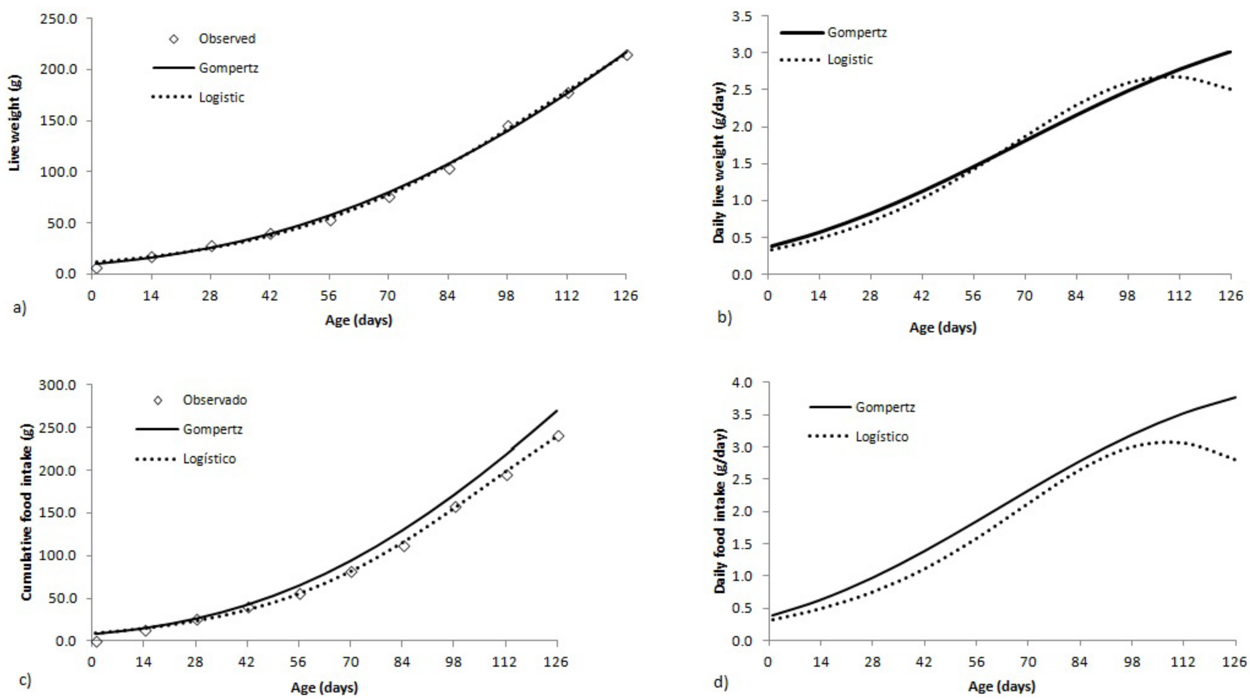

Figure 1 – Gompertz and logistic curves for live weight (a), daily live weight (b), cumulative food intake (c), and daily food intake (d) of bullfrogs during the fattening phase.

The curves estimating cumulative intake of the commercial diet during the fattening phase of bullfrogs differed after day 42 (Figure 1c). For the logistic model, the observed values were closer to the predicted values, with this model thus being more adequate to estimate cumulative food intake. The Gompertz and logistic models overestimated the initial value (Figure 1c).

The graphic representation of the growth rate shows the daily gain (in g) in live weight (Figure 1b), liver weight (Figure 2b), fat body weight (Figure 2d), and leg weight (Figure 2f). The Gompertz curves were only ascending, whereas the logistic curves show the day when the growth rate was maximum for live weight (109 days, figure 1b), liver weight (105 days, figure 2b), fat body weight (105 days, figure 2c), and leg weight (109 days, figure 2f). This maximum growth occurred on similar days, except for the maximum growth in liver weight which occurred earlier. This finding might be explained by the numerous physiological functions of liver tissue, which must be structurally formed to perform these activities.

The growth curves obtained with the Gompertz and logistic models for live weight (Figure 1a), liver weight (Figure 2a), fat body weight (Figure 2c) and leg weight (Figure 2e) were similar to each other and close to the observed data. For cumulative food intake, the logistic model provided predicted values that were closer to the expected values (Figure 1c).

Comparison of observed values and values predicted with the logistic model for live weight and cumulative food intake of bullfrogs during the fattening phase confirmed the excellent fit through linear regression, with an R^2 value of 0.9985 (Figure 3a) and 0.9980 (Figure 3b), respectively.

The set of goodness-of-fit criteria adopted was satisfactory to select the logistic function as the best model. Adequate model selection criteria should be adopted since these parameters indicate the most appropriate model to describe the body growth of a population (Mendes et al. 2009; Silveira et al. 2011).

Mathematical models are used as tools to estimate the requirements of the animals and the nutritional value of foods in each scenario of agricultural production and therefore has an important role in providing information that can be used in the decision making process in a property (Rezende et al. 2011). These sets of information can improve the technical and economic efficiency of animal feed system through computer programs easy access for producers of frogs.

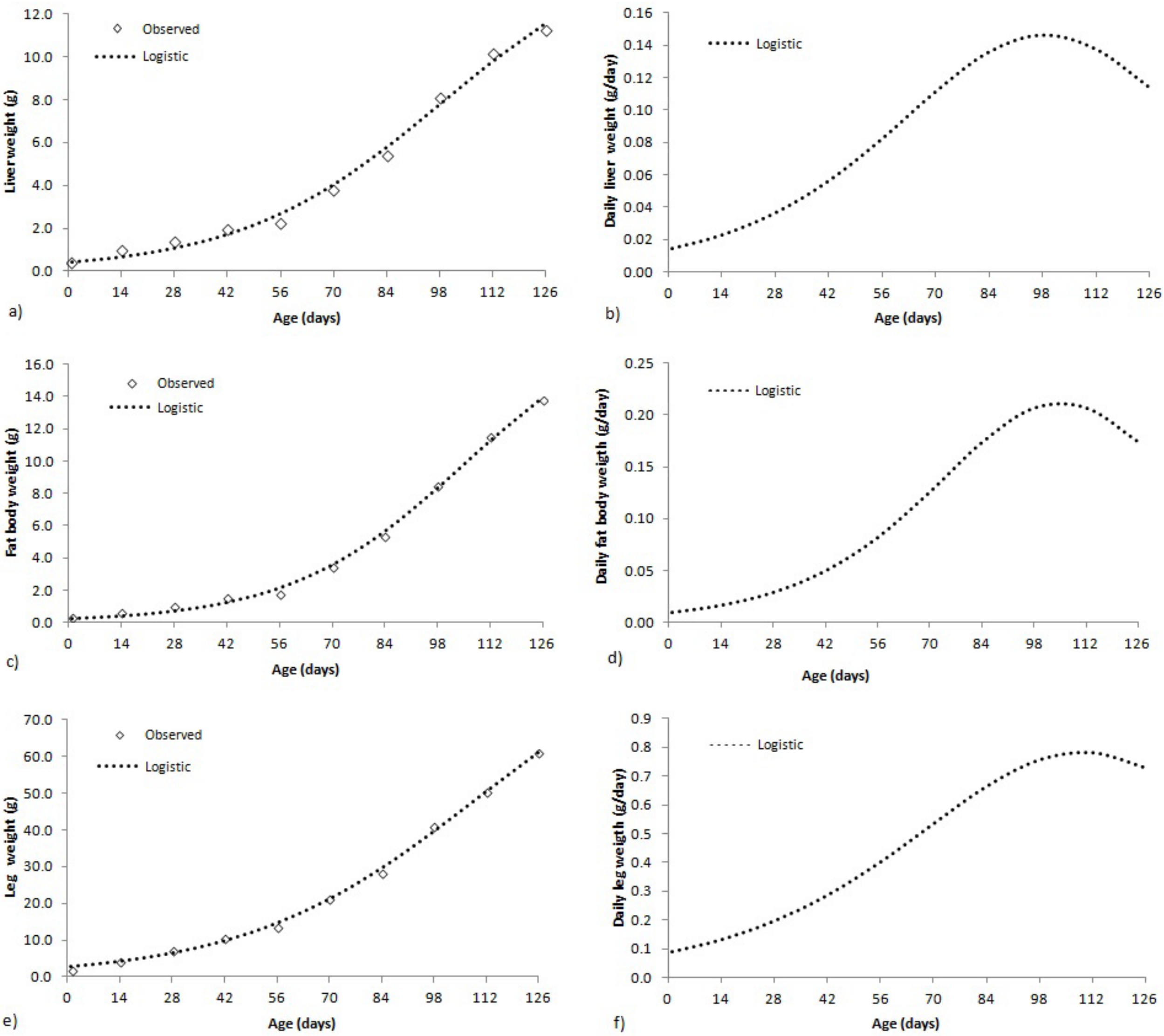

Figure 2 – Logistic curves for liver weight (a), fat body weight (c) and leg weight (e) and for daily liver (b), fat body (d) and leg (f) weight of bullfrogs during the fattening phase.

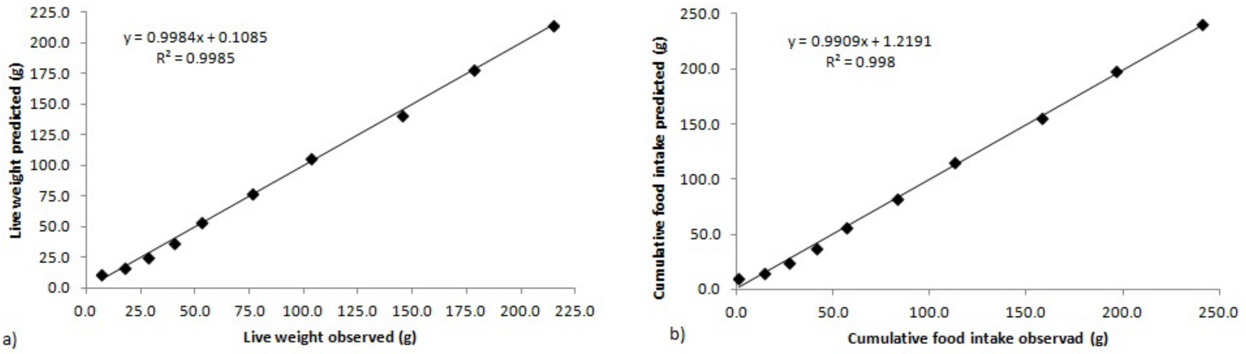

Figure 3 – Comparison of observed values and values predicted with the logistic model for live weight and cumulative food intake of bullfrogs during the fattening phase.

CONCLUSIONS

Live weight, development of liver tissue and fat body, leg growth and cumulative food intake of bullfrogs as a function of time were better estimated with the logistic model. This model may be a useful tool to describe the growth and food intake of bullfrogs.

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